(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 May 2007 (31.05.2007)

PCT

(10) International Publication Number
WO 2007/062043 A1

(51) International Patent Classification:
A61K 31/7088 (2006.01) *A61P 37/00* (2006.01)

(21) International Application Number:
PCT/US2006/045064

(22) International Filing Date:
20 November 2006 (20.11.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/739,806 23 November 2005 (23.11.2005) US

(71) Applicant *(for all designated States except US)*: **3M INNOVATIVE PROPERTIES COMPANY** [US/US]; 3M Center, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GORDEN, Keith, B.** [US/US]; 3M Center, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US). **ALKAN, Sefik, S.** [CH/US]; 3M Center, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US).

(74) Agents: **GRAM, Christopher, D.** et al.; 3M Center, Office Of Intellectual Property Counsel, Post Office Box 33427, Saint Paul, Minnesota 55133-3427 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

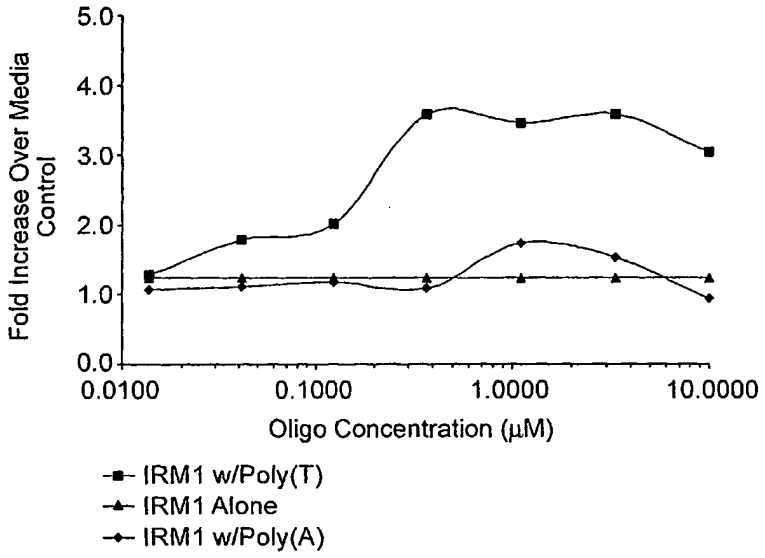
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report*

[Continued on next page]

(54) Title: METHOD OF ACTIVATING MURINE TOLL-LIKE RECEPTOR 8



(57) Abstract: The present invention provides a method of activation murine TLR8. Generally, the method includes contacting a cell expressing the murine TLR8 with a first IRM compound that comprises a TLR8 agonist, and contacting the cell expressing the murine TLR8 with a second IRM compound, wherein the second IRM compound comprises an oligonucleotide sequence comprising at least seven bases in length wherein at least one base is a thymine or a uracil.

WO 2007/062043 A1

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHOD OF ACTIVATING MURINE TOLL-LIKE RECEPTOR 8

Background

There has been a major effort in recent years, with significant success, to discover new drug compounds that act by stimulating certain key aspects of the immune system, as well as by suppressing certain other aspects (see, e.g., U.S. Pat. Nos. 6,039,969 and 6,200,592). These compounds, referred to herein as immune response modifiers (IRMs), appear to act through basic immune system mechanisms known as Toll-like receptors (TLRs) to induce selected cytokine biosynthesis, induction of co-stimulatory molecules, and increased antigen-presenting capacity.

Many of the IRM compounds are small organic molecule imidazoquinoline amine derivatives (see, e.g., U.S. Pat. No. 4,689,338), but a number of other compound classes are known as well (see, e.g., U.S. Pat. Nos. 5,446,153; 6,194,425; and 6,110,929; and International Publication Number WO 2005/079195) and more are still being discovered. Other IRMs have higher molecular weights, such as oligonucleotides, including CpGs (see, e.g., U.S. Pat. No. 6,194,388).

Mice are often used as an animal model for conducting immunological experiments because, with one notable exception, murine TLRs function similarly to human TLRs. The one exception is murine TLR8. Immortalized human embryonic kidney cells (HEK293) transfected with murine TLR8 do not produce TLR8-mediated biological activity when treated with a TLR8 agonist, leading to the suggestion that murine TLR8 is non-functional. Jurk *et al.* (2002), *Nature Immunology*, 3(6):499.

In view of the great potential for IRMs as tools for basic research, and despite the important work that has already been done, there is a substantial ongoing need to develop a useful animal model for studying TLR8-mediated immune activity.

Summary

It has been found that TLR8 agonist IRM compounds can be used to activate murine TLR8 when combined with certain oligonucleotides. Accordingly, the present invention provides a method of activating murine Toll-like receptor 8 (TLR8). Generally, the method includes contacting a cell expressing murine TLR8 with a first IRM compound that comprises a TLR8 agonist; and contacting the cell expressing the murine TLR8 with a

second IRM compound, wherein the second IRM compound comprises an oligonucleotide sequence comprising at least seven bases in length wherein at least one base is a thymine or a uracil.

Various other features and advantages of the present invention should become readily apparent with reference to the following detailed description, examples, claims and appended drawings. In several places throughout the specification, guidance is provided through lists of examples. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

Brief Description of the Drawings

Fig. 1 is a line graph demonstrating murine TLR8 function after contact with a combination of a TLR8 agonist and an oligonucleotide according to one embodiment of the invention.

Fig. 2 is a bar graph showing induction of TNF-α by wild-type murine PBMCs after contact with TLR agonists and poly(T) oligonucleotide.

Fig. 3 is a bar graph showing induction of TNF-α by TLR7 deficient murine PBMCs after contact with TLR agonists and poly(T) oligonucleotide.

Detailed Description of Illustrative Embodiments of the Invention

The present invention involves activation of murine TLR8, previously considered to be non-functional. The immune systems of mice and humans are similar enough that mice are often used as animal models of the human immune system in drug discovery and development and basic immunological research. One drawback to using mice as a model of the human immune system has been the difficulty in producing detectable function from the murine version of TLR8, the human counterpart of which is an important mediator of immune function in humans. Therefore, until now, mice have been considered unsuitable experimental models for studying TLR8-mediated immune function.

The invention provides a method of activating murine Toll-like receptor 8 (TLR8). Generally, the method includes contacting a cell expressing murine TLR8 with a TLR8 agonist and an oligonucleotide comprising at least seven bases in length wherein at least one base is a thymine or a uracil. In one aspect, the invention may be employed to activate cells expressing murine TLR8 *ex vivo*. The cell expressing the murine TLR8 may

be a mouse immune cell in cell culture expressing native TLR8. Alternatively, the cell may be a homologous host cell or a heterologous host cell into which a nucleotide sequence encoding a functional portion of the murine TLR8 has been transfected. In another aspect, the invention may be employed to activate cells expressing murine TLR8 *in vivo*. In any event, the invention provides the possibility of employing mice to discover, study, and/or better understand TLR8-mediated immunological activity.

For purposes of this invention, the following terms shall have the meanings set forth as follows:

"Agonist" refers to a compound that can combine with a receptor (e.g., a TLR) to induce a biological activity. An agonist may be a ligand that directly binds to the receptor. Alternatively, an agonist may combine with a receptor indirectly by, for example, (a) forming a complex with another molecule that directly binds to the receptor, or (b) otherwise results in the modification of another compound so that the other compound directly binds to the receptor (e.g., cellular signaling). An agonist may be referred to as an agonist of a particular TLR (e.g., a TLR8 agonist) or a particular combination of TLRs (e.g., a TLR 7/8 agonist – an agonist of both TLR7 and TLR8).

"Agonist-receptor interaction" refers to any direct or indirect interaction such as, for example, binding, forming a complex, or biochemical modification that induces a cellular activity.

"Immune cell" refers to a cell of the immune system, i.e., a cell directly or indirectly involved in the generation or maintenance of an immune response, regardless of whether the immune response is innate or acquired, humoral or cell-mediated.

"Induce" and variations thereof refer to any measurable increase in biological activity. For example, induction of a particular cytokine refers to an increase in the production of the cytokine.

"IRM compound" refers generally to a compound that alters the level of one or more immune regulatory molecules, e.g., cytokines or co-stimulatory markers, when administered to an IRM-responsive cell. Representative IRM compounds include the small organic molecules, purine derivatives, small heterocyclic compounds, amide derivatives, and oligonucleotide sequences described below.

"Selective" and variations thereof refer to having a differential impact on biological activity to any degree. An agonist that selectively modulates biological activity

through a particular TLR may be a TLR-selective agonist. TLR-selectivity may be described with respect to a particular TLR (e.g., TLR8-selective) or with respect to a particular combination of TLRs (e.g., TLR 7/9-selective). A TLR selective (e.g., TLR8-selective) compound may exclusively induce biological activity mediated by the indicated TLR (i.e., TLR-specific), or may induce biological activity mediated through multiple TLRs, but induce activity mediated through the indicated TLR to a greater extent than any other TLR (i.e., TLR-dominant such as, for example, TLR8-dominant).

"smIRM" refers generally to a small molecule IRM compound, an IRM compound having a molecular weight of about 1 kilodalton (kDa) or less.

"TLR-mediated" refers to a biological activity (e.g., cytokine production) that results, directly or indirectly, from TLR function. A particular biological activity may be referred to as mediated by a particular TLR (e.g., "TLR8-mediated").

Also herein, the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

The TLR agonism for a particular compound may be assessed in any suitable manner. For example, assays and recombinant cell lines suitable for detecting TLR agonism of test compounds are described, for example, in U.S. Patent Publication Nos. US2004/0014779, US2004/0132079, US2004/0162309, US2004/0171086, US2004/0191833, and US2004/0197865.

Regardless of the particular assay employed, a compound can be identified as an agonist of a particular TLR if performing the assay with a compound results in at least a threshold increase of some biological activity mediated by the particular TLR. Conversely, a compound may be identified as not acting as an agonist of a specified TLR if, when used to perform an assay designed to detect biological activity mediated by the specified TLR, the compound fails to elicit a threshold increase in the biological activity. Unless otherwise indicated, an increase in biological activity refers to an increase in the same biological activity over that observed in an appropriate control. An assay may or may not be performed in conjunction with the appropriate control. With experience, one skilled in the art may develop sufficient familiarity with a particular assay (e.g., the range of values observed in an appropriate control under specific assay conditions) that performing a control may not always be necessary to determine the TLR agonism of a compound in a particular assay.

The precise threshold increase of TLR-mediated biological activity for determining whether a particular compound is or is not an agonist of a particular TLR in a given assay may vary according to factors known in the art including but not limited to the biological activity observed as the endpoint of the assay, the method used to measure or detect the endpoint of the assay, the signal-to-noise ratio of the assay, the precision of the assay, and whether the same assay is being used to determine the agonism of a compound for two or more TLRs. Accordingly it is not practical to set forth generally the threshold increase of TLR-mediated biological activity required to identify a compound as being an agonist or a non-agonist of a particular TLR for all possible assays. Those of ordinary skill in the art, however, can readily determine the appropriate threshold with due consideration of such factors.

Assays employing HEK293 cells transfected with an expressible TLR structural gene may use a threshold of, for example, at least a three-fold increase in a TLR-mediated biological activity (e.g., NFκB activation) when the compound is provided at a concentration of, for example, from about 1 μM to about 10 μM for identifying a compound as an agonist of the TLR transfected into the cell. However, different thresholds and/or different concentration ranges may be suitable in certain circumstances. Also, different thresholds may be appropriate for different assays.

Exemplary TLR8-mediated biological activities that may result from activating murine TLR8 can include, for example, induction of co-stimulatory marker expression (e.g., CD40, CD80, CD86, etc.), induction of surface marker expression (e.g., CCR7), activation of NF-κB, induction of an intercellular adhesion molecule (ICAM, e.g., ICAM-1, ICAM-2, I-CAM-3, etc.), increased antigen-presenting capability, maturation of myeloid dendritic cells (mDCs), and induction of certain cytokines. Cytokines induced by a TLR8-mediated biological activity include, for example, TNF-α, a Type I interferon (e.g., IFN-α, IFN-β, IFN-ω, etc.), IFN-γ, IL-1, IL-6, IL-8, IL-10, IL-12, MIP-1, MCP-1, or any combination thereof.

As noted above, the method generally includes contacting a cell expressing murine TLR8 with a first IRM compound that comprises a TLR8 agonist; and contacting the cell expressing the murine TLR8 with a second IRM compound, wherein the second IRM compound comprises an oligonucleotide comprising at least seven bases in length wherein at least one base is a thymine or a uracil.

The TLR8 agonist may be any compound capable, or potentially capable when administered in combination with the second IRM compound, of inducing at least one TLR8-mediated biological activity. The TLR8 agonist also may be an agonist of one or more of the other TLRs (e.g., a TLR7/8 agonist) or may be, to some extent, TLR8-selective. Suitable TLR8 agonists are among the IRM compounds described in detail below.

Certain IRMs are small organic molecules (e.g., molecular weight under about 1000 Daltons, preferably under about 500 Daltons, as opposed to large biological molecules such as proteins, peptides, nucleic acids, and the like) such as those disclosed in, for example, U.S. Patent Nos. 4,689,338; 4,929,624; 5,266,575; 5,268,376; 5,346,905; 5,352,784; 5,389,640; 5,446,153; 5,482,936; 5,756,747; 6,110,929; 6,194,425; 6,331,539; 6,376,669; 6,451,810; 6,525,064; 6,541,485; 6,545,016; 6,545,017; 6,573,273; 6,656,938; 6,660,735; 6,660,747; 6,664,260; 6,664,264; 6,664,265; 6,667,312; 6,670,372; 6,677,347; 6,677,348; 6,677,349; 6,683,088; 6,756,382; 6,797,718; 6,818,650; and 7,7091,214; U.S. Patent Publication Nos. 2004/0091491; 2004/0176367; and 2006/0100229; and International Publication Nos. WO 2005/18551, WO 2005/18556, WO 2005/20999, WO 2005/032484, WO 2005/048933, WO 2005/048945, WO 2005/051317, WO 2005/051324, WO 2005/066169, WO 2005/066170, WO 2005/066172, WO 2005/076783, WO 2005/079195, WO 2005/094531, WO 2005/123079, WO 2005/123080, WO 2006/009826, WO 2006/009832, WO 2006/026760, WO 2006/028451, WO 2006/028545, WO 2006/028962, WO 2006/029115, WO 2006/038923, WO 2006/065280, WO 2006/074003, WO 2006/083440, WO 2006/086449, WO 2006/091394, WO 2006/086633, WO 2006/086634, WO 2006/091567, WO 2006/091568, WO 2006/091647, WO 2006/093514, and WO 2006/098852.

Additional examples of small molecule IRMs include certain purine derivatives (such as those described in U.S. Patent Nos. 6,376,501, and 6,028,076), certain imidazoquinoline amide derivatives (such as those described in U.S. Patent No. 6,069,149), certain imidazopyridine derivatives (such as those described in U.S. Patent No. 6,518,265), certain benzimidazole derivatives (such as those described in U.S. Patent 6,387,938), certain derivatives of a 4-aminopyrimidine fused to a five membered nitrogen containing heterocyclic ring (such as adenine derivatives described in U. S. Patent Nos. 6,376,501; 6,028,076 and 6,329,381; and in WO 02/08905), certain 3-β-D-

ribofuranosylthiazolo[4,5-d]pyrimidine derivatives (such as those described in U.S. Publication No. 2003/0199461), and certain small molecule immuno-potentiator compounds such as those described, for example, in U.S. Patent Publication No. 2005/0136065.

Other IRMs include large biological molecules such as oligonucleotide sequences. Some IRM oligonucleotide sequences contain cytosine-guanine dinucleotides (CpG) and are described, for example, in U.S. Patent Nos. 6,194,388; 6,207,646; 6,239,116; 6,339,068; and 6,406,705. Some CpG-containing oligonucleotides can include synthetic immunomodulatory structural motifs such as those described, for example, in U.S. Patent Nos. 6,426,334 and 6,476,000. Other IRM nucleotide sequences lack CpG sequences and are described, for example, in International Patent Publication No. WO 00/75304. Still other IRM nucleotide sequences include guanosine- and uridine-rich single-stranded RNA (ssRNA) such as those described, for example, in Heil *et al.*, *Science*, vol. 303, pp. 1526-1529, March 5, 2004.

Other IRMs include biological molecules such as aminoalkyl glucosaminide phosphates (AGPs) and are described, for example, in U.S. Patent Nos. 6,113,918; 6,303,347; 6,525,028; and 6,649,172.

Unless otherwise indicated, reference to a compound can include the compound in any pharmaceutically acceptable form, including any isomer (e.g., diastereomer or enantiomer), salt, solvate, polymorph, and the like. In particular, if a compound is optically active, reference to the compound can include each of the compound's enantiomers as well as racemic and scalemic mixtures of the enantiomers.

IRM compounds suitable for use as the first IRM compound can include any compound that can act as an agonist of TLR8. Suitable TLR8 agonist IRM compounds may be identified from among the purine derivatives, imidazoquinoline amide derivatives, benzimidazole derivatives, adenine derivatives, aminoalkyl glucosaminide phosphates, and oligonucleotide sequences described above. Because some oligonucleotide sequences may possess TLR8 agonist activity, it is evident that some IRM compounds may possess the activities of both the first IRM compound and the second IRM compound according to the invention and, therefore, may be considered either the first IRM compound or the second IRM compound, depending upon the identity and/or activity possessed by the other IRM compound of the combination.

The TLR8 agonist may be an agonist of one or more of the other TLRs (e.g., TLR7, a TLR7/8 agonist). Alternatively, the TLR8 agonist may be a TLR8-selective agonist. As used herein, "TLR8-selective" refers to any compound that acts as an agonist of TLR8, but does not act as an agonist of TLR7.

In some embodiments of the present invention, the first IRM compound may include a 2-aminopyridine fused to a five membered nitrogen-containing heterocyclic ring, or a 4-aminopyrimidine fused to a five membered nitrogen-containing heterocyclic ring. Such compounds include, for example, imidazoquinoline amines including but not limited to substituted imidazoquinoline amines such as, for example, amide substituted imidazoquinoline amines, sulfonamide substituted imidazoquinoline amines, urea substituted imidazoquinoline amines, aryl ether substituted imidazoquinoline amines, heterocyclic ether substituted imidazoquinoline amines, amido ether substituted imidazoquinoline amines, sulfonamido ether substituted imidazoquinoline amines, urea substituted imidazoquinoline ethers, thioether substituted imidazoquinoline amines, hydroxylamine substituted imidazoquinoline amines, oxime substituted imidazoquinoline amines, 6-, 7-, 8-, or 9-aryl, heteroaryl, heterocyclyl, aryloxy or arylalkyleneoxy substituted imidazoquinoline amines, and imidazoquinoline diamines; tetrahydroimidazoquinoline amines including but not limited to amide substituted tetrahydroimidazoquinoline amines, sulfonamide substituted tetrahydroimidazoquinoline amines, urea substituted tetrahydroimidazoquinoline amines, aryl ether substituted tetrahydroimidazoquinoline amines, heterocyclic ether substituted tetrahydroimidazoquinoline amines, amido ether substituted tetrahydroimidazoquinoline amines, sulfonamido ether substituted tetrahydroimidazoquinoline amines, urea substituted tetrahydroimidazoquinoline ethers, thioether substituted tetrahydroimidazoquinoline amines, hydroxylamine substituted tetrahydroimidazoquinoline amines, oxime substituted tetrahydroimidazoquinoline amines, and tetrahydroimidazoquinoline diamines; imidazopyridine amines including but not limited to amide substituted imidazopyridine amines, sulfonamide substituted imidazopyridine amines, urea substituted imidazopyridine amines, aryl ether substituted imidazopyridine amines, heterocyclic ether substituted imidazopyridine amines, amido ether substituted imidazopyridine amines, sulfonamido ether substituted imidazopyridine amines, urea substituted imidazopyridine ethers, and thioether substituted imidazopyridine amines; 1,2-bridged imidazoquinoline amines; 6,7-

fused cycloalkylimidazopyridine amines; imidazonaphthyridine amines; tetrahydroimidazonaphthyridine amines; oxazoloquinoline amines; thiazoloquinoline amines; oxazolopyridine amines; thiazolopyridine amines; oxazolonaphthyridine amines; thiazolonaphthyridine amines; pyrazolopyridine amines; pyrazoloquinoline amines; tetrahydropyrazoloquinoline amines; pyrazolonaphthyridine amines; tetrahydropyrazolonaphthyridine amines; and 1*H*-imidazo dimers fused to pyridine amines, quinoline amines, tetrahydroquinoline amines, naphthyridine amines, or tetrahydronaphthyridine amines.

In certain embodiments, the IRM compound may be an imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, a thiazolonaphthyridine amine, a pyrazolopyridine amine, a pyrazoloquinoline amine, a tetrahydropyrazoloquinoline amine, a pyrazolonaphthyridine amine, or a tetrahydropyrazolonaphthyridine amine.

In certain embodiments, the IRM compound may be a substituted imidazoquinoline amine, a tetrahydroimidazoquinoline amine, an imidazopyridine amine, a 1,2-bridged imidazoquinoline amine, a 6,7-fused cycloalkylimidazopyridine amine, an imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, a thiazolonaphthyridine amine, a pyrazolopyridine amine, a pyrazoloquinoline amine, a tetrahydropyrazoloquinoline amine, a pyrazolonaphthyridine amine, or a tetrahydropyrazolonaphthyridine amine.

As used herein, a substituted imidazoquinoline amine refers to an amide substituted imidazoquinoline amine, a sulfonamide substituted imidazoquinoline amine, a urea substituted imidazoquinoline amine, an aryl ether substituted imidazoquinoline amine, a heterocyclic ether substituted imidazoquinoline amine, an amido ether substituted imidazoquinoline amine, a sulfonamido ether substituted imidazoquinoline amine, a urea substituted imidazoquinoline ether, a thioether substituted imidazoquinoline amine, a hydroxylamine substituted imidazoquinoline amine, an oxime substituted imidazoquinoline amine, a 6-, 7-, 8-, or 9-aryl, heteroaryl, heterocyclyl, aryloxy or arylalkyleneoxy substituted imidazoquinoline amine, or an imidazoquinoline diamine. As used herein, substituted imidazoquinoline amines specifically and expressly exclude 1-(2-

methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine and 4-amino-α,α-dimethyl-2-ethoxymethyl-1*H*-imidazo[4,5-*c*]quinolin-1-ethanol.

In certain embodiments, the TLR8 agonist may be a tetrahydroimidazoquinoline amine such as, for example, 4-amino-2-(ethoxymethyl)-α,α-dimethyl-6,7,8,9-tetrahydro-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol. In other embodiments, the TLR8 agonist may be a thiazoloquinoline amine such as, for example, 2-propylthiazolo[4,5-*c*]quinolin-4-amine or *N*-[3-(4-amino-2-propylthiazolo[4,5-*c*]quinolin-7-yl)phenyl]methanesulfonamide. In other embodiments, the TLR8 agonist may be an imidazoquinoline diamine such as, for example, 1-(2-amino-2-methylpropyl)-2-(ethoxymethyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine. In other embodiments, the TLR8 agonist may be an imidazoquinoline amine such as, for example, 1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine.

In still other embodiments, the TLR8 agonist may be a guanine- and/or uridine-rich single-stranded RNA (ssRNA) such as, for example, RNA40 (GCCCGUCUGUUGUGUGACUC) or RNA42 (ACCCAUCUAUUAUAUAACUC) described in Heil *et al.*, *Science*, vol. 303, pp. 1526-1529, March 5, 2004 .

The second IRM compound may be any suitable oligonucleotide sequence – e.g., an oligonucleotide sequence comprising at least seven bases in length wherein at least one base is a thymine or a uracil. In some embodiments, a suitable immunostimulatory oligonucleotide may contain CpG ODN sequences such as, for example, CpG-A ODN, CpG-B ODN, or CpG-C ODN sequences. However, other oligonucleotide sequences may be suitable as well. In many embodiments, the oligonucleotide contains at least residue that is a thymine or a uracil. For example, a poly(T) 13-mer oligonucleotide has been identified as being capable of enhancing TLR8-mediated biological activity (Fig. 1).

In some embodiments, the oligonucleotide can be at least five bases in length such as, for example, at least seven bases in length, at least 13 bases in length, at least 26 bases in length, at least 30 bases in length or at least 45 bases in length. In some embodiments, the oligonucleotide may be no more than 30 bases in length such as, for example, no more than 26 bases in length, no more than 13 bases in length, or no more than eight bases in length. Thus, a suitable oligonucleotide may be, for example, from five to 13 bases in length, from seven to 45 bases in length, from 13 to 30 bases in length, from seven to 26 bases in length, etc.

The combination of the TLR8 agonist and oligonucleotide sequence may be provided in a single formulation. In other cases, the combination of the TLR8 agonist and the oligonucleotide may be provided in separate formulations. When provided in separate formulations, the TLR8 agonist portion of the combination and the oligonucleotide portion of the combination may be provided in any order. Consequently, designation of a particular IRM compound as the first IRM compound or the second IRM compound refers to their respective functional activity as part of the combination (i.e., TLR8 agonist vs. oligonucleotide) and does not expressly or implicitly require or suggest any particular order to contact with the cells that express murine TLR8.

The TLR8 agonist and oligonucleotide sequence may be provided in any formulation or combination of formulations suitable, as the case may be, for contacting cells expressing murine TLR8, either *ex vivo* (i.e., in cell culture) or *in vivo* (i.e., administration to a mouse). Suitable types of formulations are described, for example, in U.S. Pat. No. 5,736,553; U.S. Pat. No. 5,238,944; U.S. Pat. No. 5,939,090; U.S. Pat. No. 6,365,166; U.S. Pat. No. 6,245,776; U.S. Pat. No. 6,486,186; European Patent No. EP 0 394 026; and International Patent Publication No. WO 03/045391. A formulation may be provided in any suitable form including, but not limited to, a solution, a suspension, an emulsion, or any form of mixture. A formulation may include any pharmaceutically acceptable excipient, carrier, or vehicle. For example, a formulation may be delivered in a conventional dosage form such as, for example, a cream, an ointment, an aerosol formulation, a non-aerosol spray, a gel, a lotion, a tablet, an elixir, and the like. A formulation may further include one or more additives including but not limited to adjuvants, skin penetration enhancers, colorants, flavorings, fragrances, moisturizers, thickeners, and the like.

A formulation may be administered in any suitable manner such as, for example, non-parenterally or parenterally. As used herein, non-parenterally refers to administration through the digestive tract, including by oral ingestion. Parenterally refers to administration other than through the digestive tract such as, for example, intravenously, intramuscularly, transdermally, subcutaneously, transmucosally (e.g., by inhalation), or topically.

The composition of a formulation suitable for practicing the invention may vary according to factors known in the art including but not limited to the physical and

chemical nature of the TLR8 agonist and the oligonucleotide sequence, the nature of the carrier, the intended dosing regimen, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), the method or methods of administering the TLR8 agonist and the oligonucleotide sequence, and the species to which the formulation is being administered. Accordingly, it is not practical to set forth generally the composition of a formulation or formulations effective for all possible applications. Those of ordinary skill in the art, however, can readily determine an appropriate formulation with due consideration of such factors.

In some embodiments, a suitable formulation may include, for example, from about 0.0001% to about 10% TLR8 agonist, although in some embodiments the TLR8 agonist may be administered using a formulation that provides TLR8 agonist in a concentration outside of this range. In certain embodiments, the method includes administering to a subject a formulation that includes from about 0.01% to about 5% TLR8 agonist, for example, a formulation that includes from about 0.1 % to about 0.5% TLR8 agonist.

In some embodiments, a suitable formulation may include, for example, from about 0.0001% to about 10% oligonucleotide sequence, although in some embodiments the formulation may include oligonucleotide sequence in a concentration outside of this range. For example, a formulation may include from about 0.01% to about 1% oligonucleotide sequence.

An amount of TLR8 agonist that is effective for practicing the invention is an amount that, in combination with an oligonucleotide sequence, is capable of inducing a detectable level of at least one TLR8-mediated biological activity from a cell expressing murine TLR8. Thus, in some cases, a TLR8 agonist may be provided in an amount that, if administered without the oligonucleotide sequence, may not induce detectable TLR8-mediated biological activity from a cell expressing murine TLR8, but is capable of inducing detectable TLR8-mediated biological activity from a cell expressing murine TLR8 when provided with the oligonucleotide sequence.

In some embodiments, the methods of the present invention include administering sufficient TLR8 agonist to provide a dose of, for example, from about 100 ng/kg to about 50 mg/kg to the subject, although in some embodiments the methods may be performed by administering the TLR8 agonist in a dose outside this range. In some of these

embodiments, the method includes administering sufficient TLR8 agonist to provide a dose of from about 10 μg/kg to about 5 mg/kg to the subject, for example, a dose of from about 100 μg/kg to about 1 mg/kg.

An amount of an oligonucleotide sequence effective for practicing the invention is an amount sufficient, in combination with the TLR8 agonist, to induce a detectable level of at least one TLR8-mediated biological activity from a cell expressing murine TLR8. The precise amount of oligonucleotide sequence required to be effective may vary according to factors known in the art such as, for example, the physical and chemical nature of the TLR8 agonist and the oligonucleotide sequence, the nature of the carrier, the intended dosing regimen, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), the method or methods of administering the TLR8 agonist and the oligonucleotide sequence, the potency of the TLR8 agonist being administered with the oligonucleotide sequence, and the species to which the formulation is being administered. Accordingly, it is not practical to set forth generally the amount that constitutes an amount of oligonucleotide sequence effective for all possible applications. Those of ordinary skill in the art, however, can readily determine the appropriate amount with due consideration of such factors.

In some embodiments, the methods of the present invention include administering sufficient oligonucleotide sequence to provide a dose of, for example, from about 100 ng/kg to about 50 mg/kg to the subject, although in some embodiments the methods may be performed by administering the oligonucleotide sequence in a dose outside this range. In some of these embodiments, the method includes administering sufficient oligonucleotide sequence to provide a dose of from about 10 μg/kg to about 5 mg/kg to the subject, for example, a dose of from about 100 μg/kg to about 1 mg/kg.

The methods of the present invention may be performed on any suitable subject or cell population expressing murine TLR8. Suitable subjects include but are not limited to mice and/or murine cells in cell culture. Suitable subjects also include heterologous cells into which a nucleotide sequence encoding at least a functional portion of murine TLR8 has been cloned, including, for example, bacteria, fungi, human, non-human primate, other mammalian, other animal, or plant cells.

Examples

The following examples have been selected merely to further illustrate features, advantages, and other details of the invention. It is to be expressly understood, however, that while the examples serve this purpose, the particular materials and amounts used as well as other conditions and details are not to be construed in a matter that would unduly limit the scope of this invention.

IRM Compounds

The IRM compounds used in the examples are shown in Table 1.

Table 1

Compound	Chemical Name	Reference
IRM1	4-amino-2-(ethoxymethyl)-α,α-dimethyl-6,7,8,9-tetrahydro-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol	U.S. 5,352,784 Example 91
IRM2	2-propylthiazolo[4,5-*c*]quinolin-4-amine	U.S. 6,110,929 Example 12
IRM3	N-{2-[4-amino-2-(ethoxymethyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl]-1,1-dimethylethyl}methanesulfonamide	U.S. 6,677,349 Example 268
IRM4	1-(2-amino-2-methylpropyl)-2-(ethoxymethyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine	U.S. 6,677,349 Example 268 Part G
IRM5	1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine	U.S. 4,689,338 Example 99

Cells

HEK293 cells - immortalized human embryonic kidney cells, available from American Type Culture Collection, Manassas, VA, ATCC No. CRL-1573.

Cell Culture Media/Buffers

Complete RPMI was prepared by mixing RPMI 1640 with 25mM HEPES, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, and 1 mM L-glutamine (Celox Laboratories, Inc., Minneapolis, MN) supplemented with 10% heat inactivated fetal calf serum (FCS) (Hyclone Laboratories, Inc., Logan, UT) and 1% penicillin/streptomycin (Sigma Chemical Co., St. Louis, MO).

IGEN PBS Buffer was prepared from Dulbecco's Phosphate Buffered Saline without calcium or magnesium (DPBS, Biosource International, Camarillo, CA), with 0.5% bovine serum albumin (BSA), 0.2% Tween, and 0.05% azide.

Example 1 - Expression of TLR8 in HEK293 cells

HEK293 cells were cultured in 90% Minimum Essential Medium (MEM) with 2 mM L-glutamine and Earle's Balanced Salt Solution (Invitrogen Corp., Rockville, MD) adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate; 10% heat-inactivated fetal calf serum. The cells were incubated at 37°C, 8% CO_2.

Twenty-four hours before transfection, HEK293 cells were adhered to a 10 cm dish (Corning 430167, Corning Inc., Corning, NY) at 37°C, 8% CO_2. The cells were co-transfected with (1) pIRES (BD Biosciences Clontech, Palo Alto, CA) either (a) unmodified (HEK293-vector) or (b) containing murine TLR8 (HEK293-TLR8), and (2) NFkB-luc reporter (Stratagene, La Jolla, CA) in a 10:1 ratio with Fugene 6 transfection reagent (Roche Diagnostics Corp., Indianapolis, IN) following the manufacturer's instructions. The plates were incubated for 24 hours following transfection and then selected in G-418 (400 μg/mL) for two weeks. The G-418-resistant cells containing either the TLR8 or empty vector were expanded in HEK293 media supplemented with G-418 for stimulation experiments.

The transfected cells were plated in white opaque 96 well plates (Costar 3917, Corning Inc., Corning, NY) at a concentration of 5 $x10^4$ cells per well in 100 μL of HEK293 media and incubated at 37°C, 8% CO_2 for 4 hours. The cells were stimulated with IRM1 at a final IRM concentration of 10 μM, either alone or in combination with oligonucleotide. Oligonucleotide was provided as poly(A) or poly(T) 13-mer oligonucleotides at a final concentration of either 10 μM, or one of six serial three-fold dilutions (to 0.014 μM). The plates were then incubated an additional 16 hours at 37°C, 5% CO_2. Luminescence was measured on an LMAX luminometer (Molecular Devices Corp., Sunnyvale, CA). Results are shown in Table 2 and Figure 1.

Table 2

	Fold Increase Over Control	
Oligonucleotide concentration (μM)	Poly(A)	Poly(T)
10.0	0.9	3.0
3.3	1.5	3.6
1.1	1.7	3.5
0.37	1.1	3.6
0.12	1.2	2.0
0.041	1.1	1.8
0.014	1.1	1.3

Example 2

Cells are prepared as described in Example 1, except that they are stimulated with either 3 μM or 10 μM IRM2, IRM3, or IRM4. TLR8 is activated in a manner dependent upon the concentration of the poly(T) oligonucleotide sequence.

Example 3

C57bl/6 mice with TLR7 knocked-out (TLR7 KO) are injected intra-peritoneally with 10, 1.0, or 0.1 milligrams per kilogram (mg/kg) of IRM1, IRM2, or IRM5 either alone or in combination with 10, 1.0, or 0.1 mg/kg of a polyT-mer oligonucleotide injected intra-peritoneally. At one, two, and four hours after administration of the IRM and/or oligonucleotide, blood is drawn from the mice and serum is collected. IFN-a, TNF, and IL-12 are measured by ELISA in the serum samples. Cytokines will be stimulated in the serum in a manner dependent upon the concentration of the IRM and oligonucleotide.

Example 4

Blood from c57bl/6 mice (WT Mouse) or 129svev / C57bl/6 TLR7 -/- mice was collected by heart puncture bleeds into EDTA vacutainer tubes. PBMCs were separated from whole blood by density gradient centrifugation using 1-STEP (Accurate Chemical and Scientific Co., NY). Blood was diluted 1:1 with Dulbecco's Phosphate Buffered

Saline (DPBS; Biosource International, Camarillo, CA). The PBMC layer was collected and washed twice with DPBS and resuspended at 4 x 10^6 cells/mL in RPMI 1640 media (Celox Laboratories, Inc., St. Paul, MN) containing 10% heat inactivated Fetal Bovine Serum (FBS; Biosource International), 1% penicillin-streptomycin (Sigma) and 50 μM 2-Mercaptoethanol (2-ME; Gibco Invitrogen, Grand Island, NY). The PBMCs were plated in 96 well sterile tissue culture plates at a concentration of 4 x 10^5 cells per well in 200 mL of the RPMI media. The plated PMBCs were stimulated with 10 μM of IRM 1, IRM2, or IRM5 either alone or in combination with poly(T) 13-mer oligonucleotide. The poly(T) oligonucleotide was provided at a final concentration of 1.1 μM, 3.3 μM, or 10 μM. The plates were incubated overnight at 37°C, 5% CO_2. Supernatants were collected and analyzed by ELISA for mouse TNF-α (Biosource International). The results of treating the wild-type c57bl/6 mice and TLR7 -/- mice are found in Figures 2 and 3, respectively

The complete disclosures of the patents, patent documents and publications cited herein are incorporated by reference in their entirety as if each were individually incorporated. In case of conflict, the present specification, including definitions, shall control.

Various modifications and alterations to this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention. Illustrative embodiments and examples are provided as examples only and are not intended to limit the scope of the present invention. The scope of the invention is limited only by the claims set forth as follows.

What is Claimed is:

1. A method of activating murine Toll-like receptor 8 (TLR8), the method comprising:

contacting a cell expressing the murine TLR8 with a first IRM compound that comprises a TLR8 agonist; and

contacting the cell expressing the murine TLR8 with a second IRM compound, wherein the second IRM compound comprises an oligonucleotide sequence comprising at least seven bases in length wherein at least one base is a thymine or a uracil.

2. The method of claim 1 wherein the first IRM compound and the second IRM compound are contacted with the cell expressing the murine TLR8 *ex vivo*.

3. The method of claim 1 wherein the cell expressing murine TLR8 is a mouse immune cell.

4. The method of claim 1 wherein the cell is a heterologous host cell.

5. The method of claim 4 further comprising cloning a nucleotide sequence encoding a functional portion of the murine TLR8 into the heterologous host cell.

6. The method of claim 1 wherein the second IRM compound comprises an oligonucleotide sequence in which at least 25% of the residues are either thymine, uracil, or a combination thereof.

7. The method of claim 6 wherein the second IRM compound comprises an oligonucleotide sequence in which at least 50% of the residues are either thymine, uracil, or a combination thereof.

8. The method of claim 7 wherein the second IRM compound comprises an oligonucleotide sequence in which at least 80% of the residues are either thymine, uracil, or a combination thereof.

9. The method of claim 8 wherein the second IRM compound comprises an oligonucleotide sequence in which at least 95% of the residues are either thymine, uracil, or a combination thereof.

10. The method of claim 1 wherein the second IRM compound comprises a CpG oligonucleotide.

11. The method of claim 1 wherein the first IRM compound comprises a TLR8-selective agonist.

12. The method of claim 1 wherein the first IRM compound and the second IRM compound are contacted with the cell expressing the murine TLR8 *in vivo*.

13. The method of claim 12 wherein the second IRM compound comprises an oligonucleotide sequence in which at least 25% of the residues are either thymine, uracil, or a combination thereof.

14. The method of claim 13 wherein the second IRM compound comprises an oligonucleotide sequence in which at least 50% of the residues are either thymine, uracil, or a combination thereof.

15. The method of claim 14 wherein the second IRM compound comprises an oligonucleotide sequence in which at least 80% of the residues are either thymine, uracil, or a combination thereof.

16. The method of claim 15 wherein the second IRM compound comprises an oligonucleotide sequence in which at least 95% of the residues are either thymine, uracil, or a combination thereof.

17. The method of claim 12 wherein the second IRM compound comprises a CpG oligonucleotide.

18. The method of claim 12 wherein the first IRM compound comprises a TLR8-selective agonist.

19. The method of claim 12 wherein the mouse comprises a TLR7 knockout mouse.

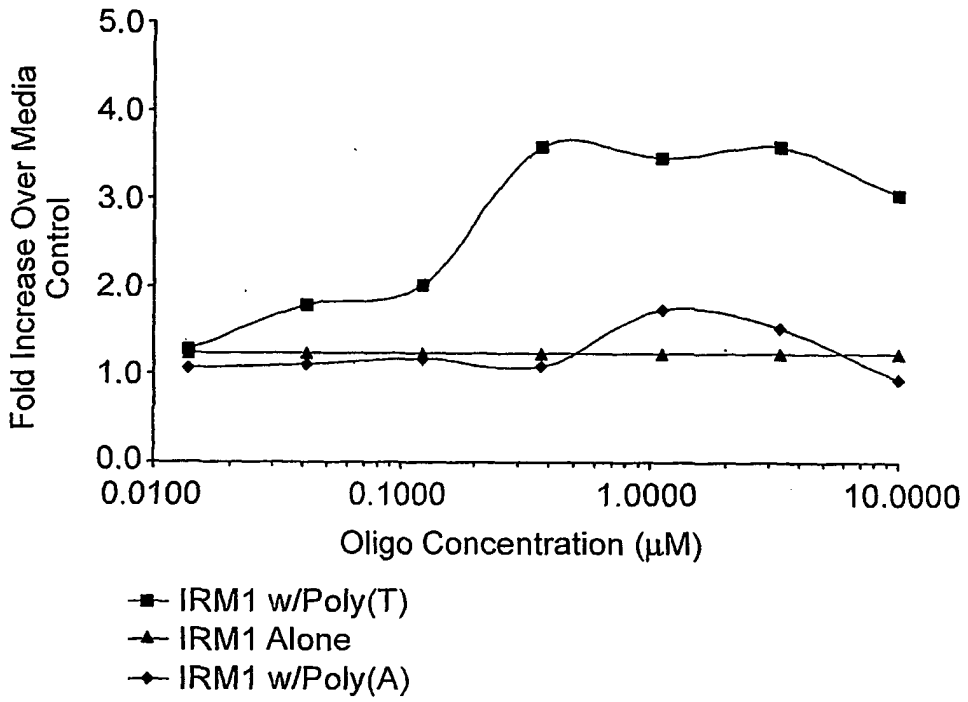

5.0
4.0
3.0
2.0
1.0
0.0
Fold Increase Over Media Control
0.0100
0.1000
1.0000
10.0000
Oligo Concentration (μM)
IRM1 w/Poly(T)
IRM1 Alone
IRM1 w/Poly(A)

FIG. 1

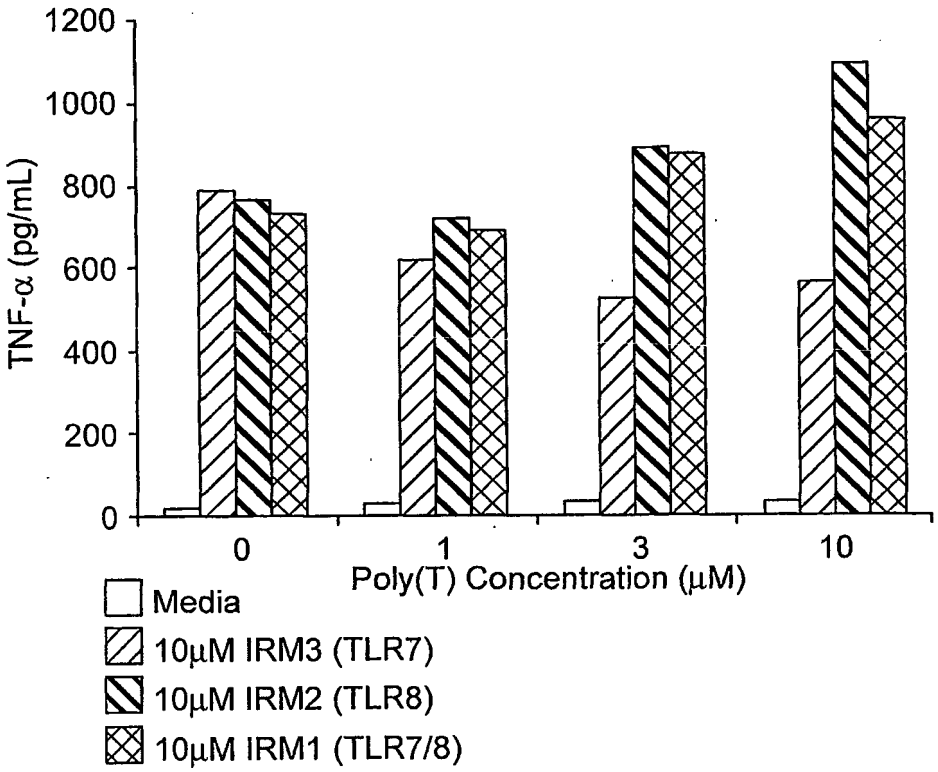

1200
1000
800
600
400
200
0
TNF-α (pg/mL)
0
1
3
10
Poly(T) Concentration (μM)
Media
10μM IRM3 (TLR7)
10μM IRM2 (TLR8)
10μM IRM1 (TLR7/8)

FIG. 2

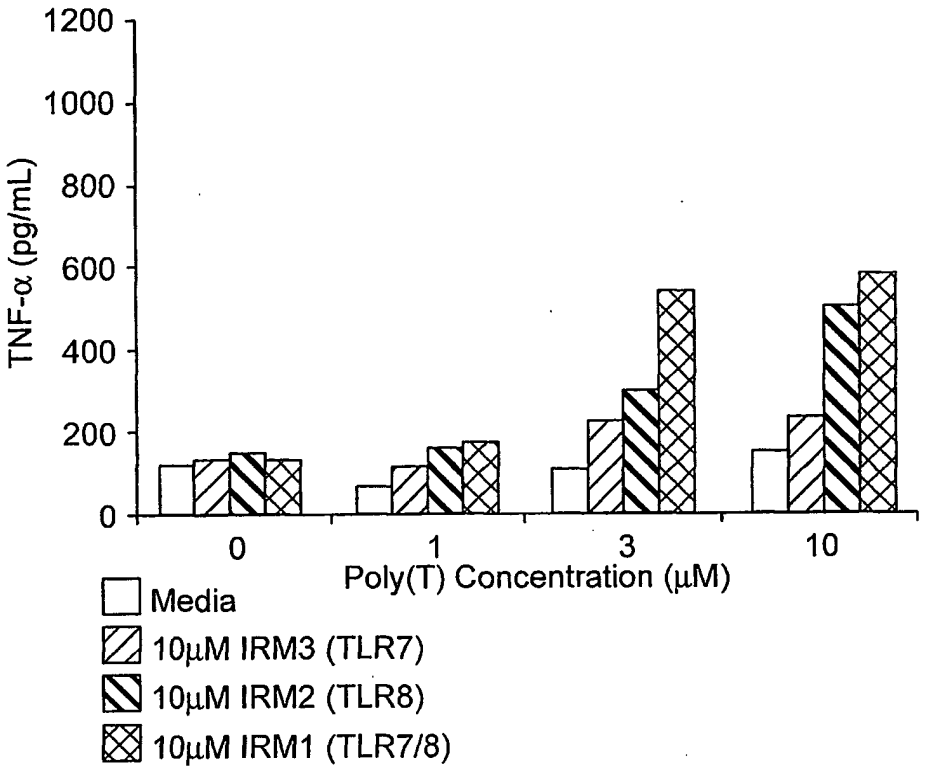

1200
1000
800
600
400
200
0
TNF-α (pg/mL)
0
1
3
10
Poly(T) Concentration (μM)
Media
10μM IRM3 (TLR7)
10μM IRM2 (TLR8)
10μM IRM1 (TLR7/8)

FIG. 3

SEQUENCE LISTING

<110> Gorden, Keith B
Alkan, Sefik S

<120> Method of Activating Murine Toll-Like Receptor 8 (TLR8)

<130> 61368WO003

<160> 4

<170> PatentIn version 3.3

<210> 1
<211> 20
<212> RNA
<213> Artificial

<220>
<223> HIV-1 U5 region nt 108-127

<400> 1
gcccgucugu ugugugacuc 20

<210> 2
<211> 20
<212> RNA
<213> Artificial

<220>
<223> G-A replacement of RNA40

<400> 2
acccaucuau uauauaacuc 20

<210> 3
<211> 13
<212> DNA
<213> Artificial

<220>
<223> poly(T) 13-mer

<400> 3
ttttttttttt 13

<210> 4
<211> 13
<212> DNA
<213> Artificial

<220>
<223> poly(A) 13-mer

<400> 4
aaaaaaaaaa aaa 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2006/045064

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/7088(2006.01)i, A61P 37/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 as above

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and Applications for Inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKIPASS, CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/086280 A2 (COLEY PHARMACEUTICAL GMBH) 23 OCTOBER 2003 see claim 1 , 33, abstract,	1-19
A	US 2003/0044429 A1 (ALAN ADEREM) 6 MARCH 2003 see claim 1, abstract	1-19

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 FEBRUARY 2007 (27.02.2007)	**27 FEBRUARY 2007 (27.02.2007)**

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701,
Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

LIM, Hea Joon

Telephone No. 82-42-481-5600

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2006/045064

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-19
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-19 pertain methods of treatment of a warm-blooded animal by therapy, and thus related to a subject matter which this International Searching Authority is not required under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search. Although claims 1-19 are directed to methods of the warm-blooded animal, the search has been carried out based on the alleged effects of the composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2006/045064

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO03086280A2	23.10.2003	AU2003230806A1	27.10.2003
		AU2003230806AA	27.10.2003
		CA2480775AA	23.10.2003
		CA2480775A1	23.10.2003
		CN1694621A	09.11.2005
		EP01499187A2	26.01.2005
		EP1499187A2	26.01.2005
		IL164354A0	18.12.2005
		JP17521749	21.07.2005
		JP18083184	30.03.2006
		JP2005521749T2	21.07.2005
		JP2006083184A2	30.03.2006
		RU2004132209A	10.06.2005
		US2003232074A1	18.12.2003
		US2003232074AA	18.12.2003
		US2006172966AA	03.08.2006
		WO2003086280A2	23.10.2003
		WO2003086280A3	25.11.2004
US20030044429A1	06.03.2003	US2005147627A1	07.07.2005
		US2005147627AA	07.07.2005